Bisphenol A, Bisphenol S, and 4-Hydroxyphenyl 4-Isoprooxyphenylsulfone (BPSIP) in Urine and Blood of Cashiers

Kristina A. Thayer, Kyla W. Taylor, Stavros Garantziotis, Shepherd Schurman, Grace E. Kissling, Dawn Hunt, Brenda Herbert, Rebecca Church, Rachael Jankowich, Mona I. Churchwell, Richard C. Scheri, Linda S. Birnbaum, and John R. Bucher

http://dx.doi.org/10.1289/ehp.1409427

Received: 3 November 2014 Accepted: 20 August 2015

**Advance Publication: 25 August 2015** 

This article will be available in a 508-conformant form upon final publication. If you require a 508-conformant version before then, please contact ehp508@niehs.nih.gov. Our staff will work with you to assess and meet your accessibility needs within 3 working days.



# Bisphenol A, Bisphenol S, and 4-Hydroxyphenyl 4-Isoprooxyphenylsulfone (BPSIP) in Urine and Blood of Cashiers

Kristina A. Thayer, <sup>1</sup> Kyla W. Taylor, <sup>1</sup> Stavros Garantziotis, <sup>2</sup> Shepherd Schurman, <sup>2</sup> Grace E. Kissling, <sup>3</sup> Dawn Hunt, <sup>2</sup> Brenda Herbert, <sup>2</sup> Rebecca Church, <sup>2</sup> Rachael Jankowich, <sup>2</sup> Mona I. Churchwell, <sup>4</sup> Richard C. Scheri, <sup>4</sup> Linda S. Birnbaum, <sup>5</sup> and John R. Bucher <sup>1</sup>

<sup>1</sup>Division of the National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, North Carolina, USA; <sup>2</sup>Clinical Research Unit, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, North Carolina, USA; <sup>3</sup>Biostatistics Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA; <sup>4</sup>Division of Biochemical Toxicology, National Center for Toxicological Research, U.S. Food & Drug Administration, Jefferson, Arkansas, USA; <sup>5</sup>National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, North Carolina, USA

**Address correspondence to** Kristina A. Thayer, National Institute of Environmental Health Sciences, P.O. Box 12233, MD K2-04, Research Triangle Park, NC 27709 USA. Telephone: (919) 541-5021. E-mail: <a href="mailto:thayer@niehs.nih.gov">thayer@niehs.nih.gov</a>

Advance Publication: Not Copyedited

Running title: BPA, BPS, and BPSIP in cashiers

**Acknowledgments:** We gratefully acknowledge the contributions of Thad Schug (NIEHS/NIH)

and Vickie Sutherland (NIEHS) for reviewing a draft version of the manuscript, to Sean Chiou

(NIEHS/NIH) for creating the figures, and to Dan Doerge (NCTR/FDA) for overseeing the

analytical chemistry work. This research was supported by the intramural research programs of

the National Institute of Environmental Health Sciences and the National Cancer Institute,

National Institutes of Health.

**Disclaimer:** The views presented in this article do not necessarily reflect those of the U.S. Food

and Drug Administration.

Competing financial interests: The authors declare they have no competing financial interests

with respect to this manuscript, or its content, or subject matter.

Advance Publication: Not Copyedited

**Abstract** 

**Background:** Bisphenol A (BPA) is a high production volume chemical associated with a wide

range of health outcomes in animal and human studies. BPA is used as a developer in thermal

paper products including cash register receipt paper; however little is known about exposure of

cashiers to BPA and alternative compounds in receipt paper.

**Objective:** To determine if handling receipt paper results in measurable absorption of BPA or the

BPA alternatives, bisphenol S (BPS) and 4-hydroxyphenyl 4-isoprooxyphenylsulfone (BPSIP).

**Methods:** Cashiers (n = 77) and non-cashiers (n=25) were recruited from the Raleigh-Durham-

Chapel Hill region of North Carolina during 2011-2013. Receipts were analysed for the presence

of BPA or alternatives considered for use in thermal paper. In cashiers, total urine and serum

BPA, BPS and BPSIP levels in post-shift samples (collected ≤ 2h after completing a shift) were

compared with pre-shift samples (collected ≥ 24 hours after a work shift). Urine levels in

cashiers were compared to levels from non-cashiers.

Results: Each receipt contained 1-2% by weight of the paper of BPA, BPS, or BPSIP. The post-

shift geometric mean total urinary BPS concentration was significantly higher than the pre-shift

mean in 33 cashiers who handled receipts containing BPS. Mean urine BPA concentrations in 31

cashiers who handled BPA receipts were as likely to decrease as increase after a shift, but the

mean post-shift concentration was significantly higher than in non-cashiers. BPSIP was detected

more frequently in urine of cashiers handling BPSIP receipts compared to non-cashiers. Only a

few cashiers had detectable levels of total BPA or BPS in serum, whereas BPSIP tended to be

detected more frequently.

**Conclusions:** Thermal receipt paper is a potential source of occupational exposure to BPA, BPS,

and BPSIP.

Advance Publication: Not Copyedited

#### Introduction

Human exposure to bisphenol A (BPA) is widespread (EFSA 2013) and BPA is associated with a wide range of health outcomes in animal and human studies (WHO 2011). Based on its use in the manufacture of polycarbonate plastic and epoxy resins in food packaging containers and can linings, the primary route of exposure to BPA in the population is thought to be oral; however, other sources of exposure have also been identified. For example, BPA and BPA analogues such as bisphenol S (BPS) are used as a dye developer in thermal paper products, including cash register receipt paper (EFSA 2013; Liao et al. 2012c; US EPA 2014). Other chemicals have been identified as theoretical alternatives to BPA in thermal paper in the US EPA Design for Environment (DfE) report "Bisphenol A alternatives in paper", such as the BPS derivative 4-hydroxyphenyl 4-isoprooxyphenylsulfone (also called BPSIP or "D-8"), although the extent to which they are being used is not known (US EPA 2014) (Figure 1). Notably, the goal of the DfE report was not to recommend a safe alternative(s) to BPA, but rather summarize information on potential hazard. If thermal paper contributes to increased uptake of BPA or its analogues, then a study of occupationally exposed individuals such as cashiers may be informative.

Very little biomonitoring data are available to determine whether cashiers, as compared to non-cashiers, have higher urine or blood levels of BPA or BPA alternatives. There are reports of higher urinary BPA levels in cashiers participating in the Health Outcomes and Measures of the Environment Study (Braun et al. 2011) and in people who reported working in retail industries in the 2003-2004 National Health and Nutrition Examination Survey (NHANES) (Lunder et al. 2010). However, neither of these studies specifically collected samples near the time of the work shift. Studies to simulate exposure in cashiers from dermal contact suggest an extensive amount of contact is needed in order to detect a post-handling increase in BPA (Ehrlich et al. 2014;

Porras et al. 2014), at least with dry hands. Wet conditions appear to facilitate skin transfer (Biedermann et al. 2010). The simulation studies only focus on dermal exposure, but other possible pathways of exposure for cashiers include hand-to-mouth ingestion after handling receipts and inhalation of dust containing the developers. Use of an ethanol-based hand sanitizers has been shown to enhance transfer of BPA from the receipt to the surface of the hand (Hormann et al. 2014).

The main objective of this study was to test the hypothesis that occupational exposure to thermal receipt paper results in increased urine and/or serum levels of BPA or its analogues in cashiers when measured shortly after they complete a work shift compared to levels measured  $\geq$  24 hours after completing a shift. We also analyzed samples of receipt paper to verify potential exposures and determine whether theoretical BPA alternatives identified in a recent report from the U.S. Environmental Protection Agency (EPA) are actually in use (US EPA 2014). We matched our analysis of biospecimens in cashiers with an analysis of a receipt paper sample provided by the cashier. Thus, we were able to evaluate the association of levels in urine and serum with detection of BPA, BPS, or BPSIP in thermal receipt paper. We also compared urine levels in cashiers to samples from non-cashiers.

#### Methods

#### Participant recruitment and selection

Cashiers (required to be aged >18 years, non-pregnant, and working at cash register for at least 20 hours a week) and non-cashiers were recruited by open advertisement from the Raleigh-Durham-Chapel Hill region of North Carolina during June 2011- September 2013. Cashiers were asked to provide proof of employment as a cashier and all participants were asked to provide

Advance Publication: Not Copyedited

medical history including disease status, current medications, alcohol and cigarette use, and food and drink consumption during the previous 24 hours (yes/no) at study enrollment. A post-shift questionnaire was administered to a subset of cashiers to assess hours worked at register, average number of transactions, consumption of food or beverage from metal containers, use of polycarbonate plastic, frequency of hand washing, and use of gloves and hand creams during work. All human subject research activities were conducted at the NIEHS Clinical Research Unit (CRU) in accordance with protocols approved by the National Institute of Environmental Health Sciences Institutional Review Board (IRB #10-E-0063) and all participants gave informed consent before providing medical history and donating samples. Participation of the National Center for Toxicological Research (NCTR) laboratory was reviewed and approved by the FDA Research Involving Human Subjects Committee (RIHSC #11-067T).

#### Receipt, blood, and urine sample collection

Care was taken to avoid BPA contamination that may come from laboratory materials and equipment by using glass pipets, polypropylene containers, including water blanks for blood and urine collecting and processing procedures, and providing special instructions to CRU staff.

Each cashier provided a receipt sample from her/his place of employment at least 12 inches long in Ziploc bags (which do not contain BPA). Two sets of samples were collected from each cashier, one "post-shift" sample collected within 2 hours of completing a work shift, and one "pre-shift" sample collected at least 24 hours after a work shift had been completed. To accommodate cashier work schedules, sample collections did not have to occur before and after the same work shift, and in ~30% of cashiers the post-shift sample was the first sample collected (see Supplemental Material, Table S1 for complete study data from each participant). We

Advance Publication: Not Copyedited

initially intended to have both visits occur on the same day but found this to present a significant challenge to participant recruitment. Thus, in order to accommodate cashier work schedules, the "pre-shift" sample was collected at the CRU at a visit that occurred after being off-duty for at least 24 hours. None of the study participants were required to fast or avoid specific food items or consumer products. A single urine sample was collected from each non-cashier at the CRU during normal CRU business hours (8 AM to 4:30 PM).

Blood samples were taken by trained phlebotomists using a metal 1", 22 gauge needle (Becton Dickinson Part #367210) attached to a disposable polypropylene tube holder (Becton Dickinson Vacutainer, Part #364815). Blood was collected into a 10 ml non-siliconized "red-top" glass blood collection tube without clot activators or other additives (Becton Dickinson Part #366441). Samples were allowed to clot at room temperature for at least 60 minutes, centrifuged at 1,200 RPM for 10 minutes, and serum transferred using glass disposable pipets (Kimble Chase, Part #63B93-P) into 1.5 ml polypropylene microcentrifuge tubes (Sarstedt, Part #76.690). Samples were stored at -80°C, shipped on dry ice to NCTR for analysis, and stored at -60°C until analyzed.

Urine samples were collected in polypropylene collection cups (Andwin Scientific, Catalogue #5050-3). Water blanks using HPLC-grade water were prepared in the same manner and collection containers as the blood and urine. One mL samples of serum, urine, and two water blanks (one for blood, one for urine) were aliquoted into four 1.5 mL polypropylene microcentrifuge tubes (Sarstedt, Part # 76.690) for storage. Samples were stored at -80°C, shipped on dry ice to NCTR for analysis, and stored at -60°C until analyzed.

Advance Publication: Not Copyedited

Materials used for analytical chemistry

All HPLC solvents including water were Optima LCMS grade purchased from Fisher Scientific

except for methanol purchased from JT Baker. Native BPA,  $\beta$ -glucuronidase/arylsulfatase (*Helix* 

pomatia, H1, 16 units/mg), <sup>13</sup>C<sub>12</sub>-BPS (>99% isotopic purity), and all other chemical reagents

were purchased from Sigma Aldrich. The <sup>13</sup>C<sub>12</sub>-BPA (>99% isotopic purity) was obtained from

Cambridge Isotope Labs, the unlabeled BPA-glucuronide (BPA-G) and <sup>13</sup>C<sub>12</sub>-BPA-G (>99%

isotopic purity) were produced and provided by the National Toxicology Program, <sup>13</sup>C<sub>6</sub>-BPS was

purchased from Toronto Research Chemicals, and BPSIP (98% purity) was purchased from AK

Scientific. Control Sprague Dawley rat serum (not filtered) was purchased from Bioreclamation

LLC (Westbury, NY) and the control urine sample came from a human volunteer in the

laboratory.

Receipt analysis

Receipts were analyzed for extractable compounds by placing a 100 mg portion of a receipt into

10 ml methanol and placed into an ultrasonic bath for 30 min. The methanol-extractable

components were evaluated using LC-UV (280 nm) and full-scan LC/MS (positive and negative

ion detection). The only methanol-extractable compounds detected were BPA, BPS, and BPSIP,

and they were identified by comparison of retention time and full-scan mass spectral data with

authentic standards for BPA and BPS (not shown). The amount of each compound present in

each receipt was then quantified using LC/MS/MS with internal standard calibration for BPA

and BPS and external standard calibration for BPSIP (<sup>13</sup>C<sub>12</sub>-BPA). Levels of detection were 0.2

mg BPA/g paper, 0.02 mg BPS/g paper, and 0.07 mg BPSIP/g paper.

8

Advance Publication: Not Copyedited

Sample Preparation

Serum

Serum samples for measurement of unconjugated and total BPA were prepared as previously

described using liquid-liquid extraction (Churchwell et al. 2014; Teeguarden et al. 2011). Each

serum sample was processed identically to measure both unconjugated and total BPS and BPSIP.

For cashier serum samples found to contain total BPA, BPS or BPSIP, the unconjugated form

was also analyzed to evaluate possible post-sampling contamination. Further evaluation of

possible BPA contamination was conducted by directly quantifying the individual conjugates,

BPA-G and BPA-S (Churchwell et al. 2014; Teeguarden et al. 2011). Serum samples from non-

cashiers were not analysed.

<u>Urine</u>

Urine samples for measurement of total and unconjugated BPA were prepared as previously

described (Churchwell et al. 2014; Teeguarden et al. 2011). The urine samples for BPS and

BPSIP measurements were prepared similarly to the sera except that acetonitrile was used in

place of methyl tert-butyl ether (MTBE). For unconjugated preparation, 100µl of urine, 100µl of

water and 50µl of internal standard were mixed in a deactivated Max Recovery vial. The vial was

briefly mixed and then shaken on a 23°C thermomixer for 10 min at 1,400 RPM. The vial was

centrifuged at 10,000 RPM for 10 min and stored in a -20°C freezer for 30 min. The acetonitrile

layer was transferred to a new deactivated vial with a Pasteur pipet and evaporated to dryness at

reduced pressure using a heated centrifugal concentrator. The sample was reconstituted

identically to the sera. For totals analysis, 100µl of urine, 100µl of enzyme (1mg/ml in 25mM

citrate buffer, pH 5), and 50µl of internal standard were gently mixed in a deactivated vial and

9

then incubated at 37°C for two hours. The remaining preparation steps were the same as for unconjugated analysis. Urine creatinine levels were determined at the Department of Laboratory Medicine, NIH Clinical Center, as a CLIA certified test using a Siemens Dimension EXL. Urine was stored at -80°C until testing.

#### Characterization and preparation of standards

Characterization of the <sup>13</sup>C<sub>12</sub>-BPA was performed as described in Teeguarden et al. (2011). LC-UV (Dionex AD20, 280nm) was used to verify the concentration of unlabeled and labeled BPS standards. A Luna analytical column (2.0 x 150mm, 3µ particle, Phenomenex) was used at a flow of 0.2 ml/min and an isocratic mobile phase consisting of 40% aqueous acetonitrile. Isotopic purity for the labeled BPS was 90% and no unlabeled BPS was detected by LC/MS/MS (<0.1%). The BPSIP was prepared from solid material and used as weighed.

Working standard and internal standard solutions for BPS and BPSIP were prepared in 50% acetonitrile / 50% water. Pools of control rat serum and spiked control rat serum or urine were prepared for use as daily quality control samples. In addition to the quality control samples, four enzyme blanks or four unconjugated blanks were also prepared with each sample set to establish background BPS and BPSIP levels from sample preparation.

#### LC/MS/MS determinations in urine and serum

#### BPA

LC and tandem MS (LC/MS/MS) were used with on-line column switching for the analysis of total and unconjugated native BPA in urine and serum as reported previously (Churchwell et al. 2014; Teeguarden et al. 2011). Levels of detection (LOD) were determined daily: BPA urine (0.07 - 0.25 ng/ml) and BPA serum (0.045 - 0.35 ng/ml). BPA conjugates were analyzed in

Advance Publication: Not Copyedited

serum to confirm positive findings of total BPA and the LODs for BPA-G and BPA-S were 0.04 and 0.06 ng/ml, respectively, for 100 µl aliquots.

**BPS** 

The liquid handling system consisted of an Acquity UPLC system (Waters, Inc.), a 1260 Infinity HPLC pump (Agilent), and an automated six port switching valve (Rheodyne). It also had a Luna C18(2) column (2.0 x 30mm, 3µ particle size, Phenomenex) installed between the binary solvent manager and the sample manager. The on-line SPE column was a Shodex ODP2 HP (2 x 50mm, macroporous particle type, ES Industries) and the HPLC column was a Shodex ODP2 HP (2 x 150mm. The analytical column was maintained at 45°C. The Acquity system was used to load 50µl of sample on the SPE column and to wash the SPE column. The Agilent pump eluted the sample components from the SPE column to the analytical column and kept a constant flow of mobile phase going into the mass spectrometer during sample loading periods. The switching valve was used to divert the column effluent to either waste or the analytical column. The sample was loaded at 0.3 ml/min for 5.0 min with 80% water / 20% methanol. After switching the divert valve, the concentrated sample zone was back flushed to the analytical column with 60% water / 40% acetonitrile at 0.2 ml/min for 2 min. At 2.1 min, a linear gradient raised the acetonitrile concentration to 90% over 10 min and then held steady from 12 to 14 min. At 14 min the gradient was reset to initial conditions. The SPE column was in-line with the analytical from 5.1 to 6.2 min. From 8.9 to 14.9 min the SPE column was cleaned with 95% methanol / 5% water. At 15 min, the Acquity gradient was reset to initial conditions. The total run time including sample loading was 22 min.

11

Advance Publication: Not Copyedited

A Xevo TQ-S triple quadrupole mass spectrometer (Waters) equipped with an ESI source was used in selected reaction monitoring mode for analysis of negative ions. Capillary voltage was 2.5 kV and the cone gas was 150 l/hr. Other MS parameters included source and desolvation temperatures of 150°C and 500°C, respectively, argon as collision gas (0.17 ml/min) and nitrogen as the desolvation gas (1000 l/hr). Two transitions were monitored for both the labeled and unlabeled BPS. A cone voltage of 45V was used for all transitions. Levels of detection (LOD) were determined daily: BPS urine (0.01 - 0.02 ng/ml) and BPS serum (0.002 - 0.01 ng/ml)

#### **BPSIP**

BPSIP was analyzed in urine and serum using the LC conditions described above for BPS. Concentrations of BPSIP were initially evaluated using <sup>13</sup>C<sub>12</sub>-BPA as a surrogate internal standard. The method performance was evaluated during the BPS validation using control and spiked matrices. The validation produced acceptable precision and accuracy ranges. However, when actual cashier sera or urine were analyzed, the method failed because of the wide range of suppression observed on the <sup>13</sup>C<sub>12</sub>-BPA that did not affect the BPSIP. Since no other suitable internal standard could be identified for quantification of BPSIP, semi-quantitative results were evaluated as either above or below the LOD: urine (0.01-0.02 ng/ml) and serum (0.005 - 0.008 ng/ml). Subsequently, all urine samples containing total BPSIP above the daily LOO (0.03-0.06 ng/ml) were quantified using the method of standard addition, where two aliquots of each sample were analyzed: one aliquot was spiked with a known amount of BPSIP matched to the target concentration, based on the value estimated from the original analysis; and the other without a spike. The control human urine sample from the laboratory volunteer was also analyzed in duplicate with standard addition to provide a background value of contamination during sample preparation. Quantification of BPSIP was obtained by dividing the area of the unspiked sample

Advance Publication: Not Copyedited

with the area of the spiked sample minus the area of the unspiked sample and multiplying by the amount of BPSIP added in ng/ml. The background value generated from the control urine was subtracted from each sample before results were reported. All serum samples contained total BPSIP below the LOQ (0.015 - 0.024 ng/ml) and were not analyzed further.

# Method Validation and Quality Control

## **BPA**

The validation of the on-line column switching LC/MS/MS method was reported previously (Teeguarden et al. 2011). Measurable responses for BPA were observed in all procedural blanks because trace level contamination by native BPA is difficult to avoid (Teeguarden et al. 2011; Twaddle et al. 2010; Ye et al. 2013). Accordingly, four replicate procedural blanks were analyzed with each sample set to determine a daily limit of blank (LOB). These samples, which consisted of water instead of serum, were subjected to the entire sample preparation process. The LOB was defined as the mean value + 2 SD of the replicates and the daily LOB was subtracted from each serum sample concentration (with enzymatic hydrolysis, 0.5-1.8 nM, without enzyme, 0.3-1.1 nM). In addition, daily limits of detection (LOD) were estimated from the amount of BPA producing a signal/noise ratio >3 above the LOB (with enzymatic hydrolysis, 0.2-1.1 nM, without enzyme, 0.1-0.4 nM). If the sample quantification value after subtraction of the LOB was not higher than the daily calculated LOD, it was reported as <LOD. Intra- and inter-day precision ranged from 0.6-5.3% relative standard deviation (RSD). Intra and inter-day accuracy ranged from 98-105%. Accuracy is defined as the percentage of how close the calculated value for a spiked control sample came to the actual known spiked amount.

Advance Publication: Not Copyedited

BPS and BPSIP

Calibration curves were generated for BPS by adding varying concentrations of unlabeled BPS while keeping the internal standard concentration constant. The curve was linear over the range of 0 to 10 ng/ml with a slope of 0.89. The serum and urine methods were validated over two days using control serum, spiked control serum and incurred study serum. The Sprague Dawley rat serum purchased from Bioreclamation was used as control serum for the BPS and BPSIP methods also. An incurred BPS study serum was prepared by adding a small amount of a previously analyzed BPS urine sample with a known total BPS level to a large volume of control serum. This sample was also spiked with a known amount of BPSIP. The use of the incurred study serum validates that the enzyme works properly for analysis of total BPS. Validation was done on 100 µl aliquots of serum and urine. Intra- and inter-day precision (relative standard deviation) ranged from 0.8-12.2%. Intra- and inter-day accuracy ranged from 93-107%. Control serum was spiked at 0.1 ng/ml for unconjugated and total analyses of 100 µl serum samples. Control urine was spiked at 0.1 ng/ml and 1.0 ng/ml for the validation of both total and unconjugated levels.

Duplicates of control serum and pooled incurred serum were analyzed with each serum sample set as quality control checks. The incurred study serum prepared for the validation was used also as an incurred serum for daily BPS and BPSIP analyses. Duplicates of control and spiked urine were analyzed with each urine sample set. In addition four replicate method blanks were analyzed with each sample set. These consisted of water in place of the serum and went through the entire sample preparation process. These samples provided a measurement of background BPS generated during sample preparation (i.e., limit of blank, LOB). The average concentration value of the replicates plus two standard deviations was subtracted from each sample

Advance Publication: Not Copyedited

concentration and the difference reported as the sample concentration. In addition, daily LODs were generated from calculating the signal to noise ratio of several different serum or urine samples with low calculated BPS or BPSIP values. Because of the wide variation of ion suppression seen between individual serums or urines a daily LOD for a signal to noise ratio of three to four was generated based on an average of these observations. If the sample value after subtraction of the background was not higher than the daily calculated LOD, it was reported as <LOD.

#### Assessment of potential BPA contamination

Samples were considered to show evidence of possible BPA contamination when high percentages of the BPA were present as unconjugated ( $\geq 20\%$ ) based on analysis with and without complete enzymatic hydrolysis. Direct analysis of individual BPA conjugates, BPA-G and BPA-S was also conducted since conjugates are the predominant species present in serum and urine after either oral (>99% of total BPA) or parenteral administration (>85% of total) (NTP 2008, Doerge et al. 2010). Samples where ≥20% of total BPA was present as unconjugated AND no BPA-G or BPA-S conjugate was detected (LOD 0.04 and 0.06 ng/ml, respectively) were classified as suspected contamination (Supplemental Materials Table S1).

#### Statistical Analysis

Results of the receipt paper analysis expressed as percentage of total paper weight showed that the receipts contained 1-2% of BPA, BPS, or BPSIP. We assigned cashiers to receipt groups based on the dominant analyte detected in the receipt paper. Post-shift urine levels of total BPA, BPS, and BPSIP in cashiers were compared to levels from pre-shift and to levels in samples collected from 25 non-cashiers. Analysis of urine BPA and BPS was quantitative while BPSIP

Advance Publication: Not Copyedited

analysis was frequency based, i.e., results are reported as either above or below the LOD, since most urine samples did not have BPSIP levels above the level of quantitation (LOO). We also conducted a frequency-based analysis (<LOD versus >LOD) of serum levels of total BPA and BPS in pre- and post-shift samples from cashiers and in a subset of cashier samples for BPSIP. Serum BPA and BPS were not measured in non-cashiers since the frequency of detection was low in cashiers.

Statistical analysis was conducted using SAS version 9.3 (SAS Institute Inc., Cary, NC, 2010). Creatinine-adjusted urine levels were natural log-transformed during statistical analysis, as they were right-skewed. When the level was below the LOD, a value of LOD/2 was used for BPA and BPS quantitation which is considered reasonable when the proportion of samples below the LOD is relatively small (<15%), as was the case in this study (Gillespie et al. 2005). We did not impute values <LOQ for urine BPSIP because many samples were below the LOD or LOQ. Paired t-tests were used to compare pre- to post-shift urine levels of BPA or BPS. Two-sample ttests were used to compare mean urine concentrations in non-cashiers to mean concentrations in BPA- or BPS-exposed cashiers, respectively. Frequency of detection data were compared between pre- and post-shift with McNemar's chi-square test and were compared between groups of participants using chi-square or Fisher's exact tests. One-sided p-values were used because we hypothesized a priori that cashiers would have higher levels/detection frequency of the developer used in the receipts they handled post-shift compared to pre-shift and that their levels/detection frequency would be higher than in non-cashiers. P-values less than 0.05 were considered statistically significant. We used stepwise regression with an entry significance level of 0.15 and an exit significance level of 0.10 to determine whether fasting status, defined as eating or drinking in the 8 hours preceding sample collection, and shift sequence (i.e., whether

the post-shift sample was collected first) were predictors of changes in BPA and BPS concentrations in urine between pre-shift and post-shift. Fasting status and shift sequence data available for most subjects and included in all models and not subject to removal from any model.

#### **Results**

Selected data from individual study participants included in this analysis are provided in Supplemental Material, Table S1.

### **Participants**

A total of 91 male and female cashiers aged 19-77 years were recruited from restaurants, grocery stores, pharmacies, clothing stores, bookstores and home improvement centers. Six were excluded because they did not complete both visits and 7 were excluded because they did not provide a receipt sample, the receipt paper was of poor physical quality and not analysable, or the sample was not thermal paper. One additional cashier was excluded because the pre-shift urine creatinine result was unusually low ("0"). Thus, a total of 77 cashiers were included in the analysis. Cashiers were grouped into receipt categories based on the dominant analyte detected in the paper (BPA = 33, BPS = 32, BPSIP = 12) (Table 1 and Table 2). Urine samples were also collected from 25 non-cashiers.

We have information on CRU visit dates and fasting status prior to sample collection for all of the cashiers and 24 of 25 non-cashiers (Supplemental Material, Table S1). The interval between collection of pre-shift and post-shift samples ranged from the same day to several months and was less than one week for almost 70% of cashiers. Most of the cashiers (62/77 pre-shift; 69/77 post-shift) and non-cashiers (20/25) did not fast in the 8 hours prior to sample collection. An

Advance Publication: Not Copyedited

insufficient number of cashier participants (27 of 77) completed a separate post-shift questionnaire to support quantitative analysis of factors such as length of shift, average number of transactions during the shift, consumption of metal canned foods or drinks, use of polycarbonate food packaging, use of gloves and hand creams, and degree of hand washing.

# Receipt samples

Only one analyte was the dominant form in thermal receipt paper samples with levels of the other analytes either non-detectable or only detected in amounts < 0.1% by weight in the paper tested (Table 1).

# Urine levels of total BPA, BPS, and BPSIP

While post-shift levels of urinary BPA tended to be higher than pre-shift levels in cashiers who handled BPA receipt paper [geometric mean (SD): pre = 1.89 (3.63)  $\mu$ g/g; post = 2.76 (3.53)  $\mu g/g$ , Table 3], the difference was not statistically significant (p = 0.10). There was considerable variability within individual cashiers where post-shift urine levels were actually lower than preshift levels in almost half of cashiers handling BPA containing receipts (Figure 2 and Table S1). Post-shift urine levels of BPA in the BPA-receipt paper cashier group were significantly higher than levels in non-cashiers [geometric mean (SD): 1.25 (1.79)  $\mu g/g$ ; post-shift p < 0.001]. Urine levels of BPA in our non-cashiers samples were slightly lower than the most recent NHANES 2011-2012 data (geometric mean of 1.72 μg/g creatinine) (CDC 2015). In the step-wise regression analysis, neither shift sequence nor fasting status were significant predictor variables for differences in pre- versus post-levels of BPA (data not shown).

Post-shift levels of total urinary BPS were significantly higher than levels in pre-shift for the 32 cashiers who handled BPS receipts [geometric mean (SD) pre =  $0.23 (3.89) \mu g/g$ , post = 0.54

Advance Publication: Not Copyedited

(3.62)  $\mu g/g$ ; p < 0.001; Table 3 and Table S1]. Levels of BPS were higher in post-shift samples compared to pre-shift samples for most of the cashiers in the BPS receipt group (26/32, **Figure 2**). Neither pre-shift nor post-shift urine levels of BPS in these cashiers were significantly higher than levels in non-cashiers (geometric mean (SD): 0.41 (5.26)  $\mu g/g$ ). Neither shift sequence nor fasting status were significant predictor variables for differences in pre- versus post-levels of BPS in the step-wise regression analysis (data not shown).

In the 12 cashiers who handled BPSIP receipts, the proportion of samples with detectable BPSIP was similar in pre- and post-shift samples (10/12, 83% and 9/12, 75%, respectively; p = 0.65) (Table 3). BPSIP was detected more frequently in cashiers in the BPSIP group pre- and post-shift compared to cashiers in other receipt groups where the pre- and post-shift detection frequency ranged from 12.1 to 28.2% (p < 0.02; Table 3). BPSIP was also detected significantly less often in non-cashiers (32% (8/25); p < 0.02). BPSIP concentrations were >LOQ in 58% and 67% of pre- and post-shift samples from the BPSIP cashier group, respectively, compared with 0-16% of samples from other cashier groups and non-cashiers.

#### Serum levels of total BPA, BPS, and BPSIP

In the BPA receipt group, most cashiers had pre- and post-shift levels of total serum BPA below the LOD or LOQ (26/33, or 79%, in both pre- and post-shift samples) (Table 4). Contamination was suspected in 5 of the 6 serum samples with BPA above the LOQ in the BPA cashier group based on the sample having a relatively high fraction of total present in unconjugated form (>20%) (Table S1). The presence of BPA-G and BPA-S were confirmed in these samples containing measurable total BPA but were not observed in samples showing unconjugated percentages of total BPA (data not shown), which is also indicative of contamination. BPA was

also typically below the LOD or LOQ in cashiers in the BPS group (88% at both time points) or

BPSIP group (66% pre-shift; 100% post-shift) (Table 4).

cashiers in the BPA (14/66) and BPSIP (3/24) receipt groups.

In the BPS receipt group, serum total BPS was detected significantly more frequently in post-shift samples than in pre-shift samples (13/32 or 40.6% post-shift versus 5/32 or 15.6% pre-shift, p = 0.02). Most of the 18 samples having detectable levels in the BPS group (i.e., above the LOD) were below the LOQ. Detectable levels were also measured in serum samples from

Serum BPSIP was detected in cashiers from the BPSIP receipt group at levels between the LOD and LOQ but the detection frequency did not differ between pre- and post-shift samples (7/12 or 58.3% post-shift versus 6/12 or 50% pre-shift). BPSIP was also detected in 33–44% of samples from cashiers in the BPA and BPS receipt groups (Table 4). An additional observation is that BPSIP was more consistently detected in a greater percent of samples in cashier groups (33 to 58.3%) compared to BPA (0 to 33%) or BPS (8.3 to 40.6%).

#### **Discussion**

In aggregate, our results support occupational use of thermal paper as a source of exposure to BPA, BPS, and BPSIP. However, there was considerable within-subject variability, especially for BPA, i.e., levels were often lower in post-shift samples compared with pre-shift samples (**Figure 2**). We did not have a sufficient number of completed post-shift questionnaires to support statistical analyses on which factors might predict patterns of response (length of shift, average number of transactions during the shift, consumption of canned foods or drinks, use of polycarbonate food packaging, use of gloves and hand creams, and degree of hand washing). Based on the questionnaire data we have (completed by ~30 to 40% per group), most cashiers

Advance Publication: Not Copyedited

did not use gloves, did wash hands regularly during shift, reported infrequent use of hand creams (0-1 times during shift), and did not eat or drink often from metal food cans or polycarbonate plastic food containers. Only one cashier in the BPA receipt group reported eating or drinking multiple times from metal food can or plastic food container during their shift. Most cashiers reported engaging in one transaction every 5 or 10 minutes, but some reported more than one every minute and others reported one or less in 30 minute time periods.

Our analysis of receipt content of BPA and BPS are similar to levels reported in other studies (Biedermann et al. 2010; Geens et al. 2012; Lassen et al. 2011; Liao and Kannan 2011; Liao et al. 2012c; Lu et al. 2013; Lunder et al. 2010; Mendum et al. 2011; Östberg and Noaksson 2010; Schreder 2010). We observed one predominant compound (BPA, BPS, or BPSIP) in each sample of thermal paper receipts, suggesting that only one of the compounds was used as the primary developer for any receipt. BPA and BPS are known to be used in thermal paper (US EPA 2014; Liao et al. 2012c, but this is the first confirmed use of BPSIP, which was found in receipts collected from 12 cashiers working at two retailers.

Exposure via contact with thermal paper could occur through dermal or non-dermal routes. Dermal uptake is possible, but other potential pathways of exposure for cashiers include ingestion and inhalation of dust particles containing the compounds or inhalation if the compounds become volatile. Studies designed to model cashier exposure suggest that extensive dermal contact is needed in order to detect a post-handling increase in BPA (e.g. receipts handled continuously without gloves for 2 hours (Ehrlich et al. 2014) or tightly rubbing paper for several minutes repeatedly (Porras et al. 2014). Patterns of extensive handling of receipts are unlikely to occur routinely in cashiers where contact is more likely intermittent and last only seconds at a

time (Lassen et al. 2011), highlighting the importance of considering non-dermal exposure pathways as well. The current study focused on cashiers, but other occupations involving potentially high exposures should also be considered. For example, BPA can be found in medical apparatus thermal paper at levels similar to cash register receipts (Östberg and Noaksson 2010).

We measured detectable urine levels of BPS and BPSIP in non-cashiers. The BPS result is not surprising given that BPS has been reported in urine in the general population (Liao et al. 2012a) and can also be found in food (Liao and Kannan 2013), personal care products (Liao and Kannan 2014), dust (Liao et al. 2012b), soil sediment (Liao et al. 2012d), and other paper products such as currency, tickets, and airplane boarding passes (Liao et al. 2012c). Very little is known about uses of BPSIP outside of its use as an alternative to BPA in thermal paper (US EPA 2014). To the best of our knowledge no other study has reported information on its detection in human or environmental samples, food, or receipts. BPSIP was detected more often in the serum samples of cashiers than BPA or BPS, regardless of whether BPSIP was predominant compound in the receipts they handled (Table 4), which raises questions on whether it may be more environmentally persistent, less readily cleared from the body, or perhaps exposure is more widespread than assumed. We did not measure BPSIP or the other compounds in serum samples from non-cashiers.

Ten serum samples had quantifiable levels (>LOQ) of total BPA (Table S1). However, contamination was suspected in 7 of these samples based on a relatively high portion of the total present in unconjugated form (≥20%) and the absence of detectable BPA conjugates (BPA-G and BPA-S). Sample contamination by BPA has been widely reported, even when steps are taken to minimize potential contamination during sample collection and analysis as was done in the

Advance Publication: Not Copyedited

current study (Calafat et al. 2013; Longnecker et al. 2013; Teeguarden et al. 2013; Twaddle et al. 2010; Ye et al. 2013). In contrast, there were few indications of sample contamination in our serum BPS and BPSIP analysis, perhaps reflecting their more limited usage in laboratory materials used for sample collection, sample storage, and analytical chemistry.

There are limitations to this study. Sample sizes were small in each group and our study was not designed to discern which routes of exposure might account for the observed patterns in cashiers, i.e., dermal, oral, and/or inhalational, or rule out exposure from other sources. Furthermore, there is uncertainty about the pharmacokinetics of these compounds following dermal exposure and our sample collection within 2 hours after shift may not have been ideal for detecting peak levels. A portion of BPA may be retained in the skin following dermal contact (Demierre et al. 2012; Kaddar et al. 2008; Morck et al. 2010) and may take more than a day to be taken up through the skin into systemic circulation and eliminated via urine (Marquet et al. 2011). In another study published after ours was initiated (Ehrlich et al. 2014), the highest urine levels occurred 6-10 hours after handling of receipts, at levels approximately twice as high as when collected 2 hours post-handling. Another potential limitation is that the pre-shift visit did not necessarily occur on the same day as the post-shift visit (although most occurred during the same week) and the post-shift visit occurred prior to the pre-shift visit in ~30% of participants. We also do not know the time interval between last receipt handling and sample collection in the post-shift samples and it is possible 24 hours of not handling receipts prior to pre-shift sample collection may not be a sufficient wash out period for BPA levels to return to baseline. Nor do we know how many hours were worked during the workweek preceding the post shift visit. These factors may explain why many cashiers in the BPA receipt group had lower post-shift than pre-shift levels, i.e., other sources of exposure might have had a greater influence on urine levels

Advance Publication: Not Copyedited

than occupational exposures. We also did not attempt to limit exposures to BPA from other sources, such as food or drink. Additional studies would be needed to address these limitations.

In conclusion, our results demonstrate that thermal paper is a potential source of exposure to BPA and similar compounds in cashiers and may be a source of exposure in other occupations having frequent contact with thermal paper.

#### References

- Biedermann S, Tschudin P, Grob K. 2010. Transfer of bisphenol A from thermal printer paper to the skin. Anal Bioanal Chem 398(1):571-576.
- Braun JM, Kalkbrenner AE, Calafat AM, Bernert JT, Ye X, Silva MJ, Barr DB, Sathyanarayana S, Lanphear BP. 2011. Variability and predictors of urinary bisphenol A concentrations during pregnancy. Environ Health Perspect 119(1):131-137.
- Calafat AM, Koch HM, Swan SH, Hauser R, Goldman LR, Lanphear BP, Longnecker MP, Rudel RA, Teitelbaum SL, Whyatt RM, Wolff MS. 2013. Misuse of blood serum to assess exposure to bisphenol A and phthalates. Breast Cancer Research 15(5):403.
- Centers for Disease Control and Prevention (CDC). 2015. Fourth Report on Human Exposure to Environmental Chemicals, Updated Tables, (February 2015). Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. <a href="http://www.cdc.gov/exposurereport/">http://www.cdc.gov/exposurereport/</a> [accessed 11 March 2015].
- Churchwell MI, Camacho L, Vanlandingham M, Twaddle NC, Sepehr E, Delclos KB, Fisher JW, Doerge DR. 2014. Comparison of lifestage-dependent internal dosimetry for bisphenol A, ethinyl estradiol, a reference estrogen, and endogenous estradiol to test an estrogenic mode of action in Sprague-Dawley rats. Toxicol Sci 139(1):4-20.
- Demierre AL, Peter R, Oberli A, Bourqui-Pittet M. 2012. Dermal penetration of bisphenol A in human skin contributes marginally to total exposure. Toxicol Lett 213(3):305-308.
- Doerge DR, Twaddle NC, Woodling KA, Fisher JW. 2010. Pharmacokinetics of bisphenol A in neonatal and adult rhesus monkeys. Toxicol Appl Pharmacol 248(1):1-11.
- Ehrlich S, Calafat AM, Humblet O, Smith T, Hauser R. 2014. Handling of thermal receipts as a source of exposure to bisphenol A. J Am Med Assoc 311(8):859-860.
- European Food Safety Authority (EFSA). 2013. Public consultation on the draft opinion on bisphenol A (BPA) exposure assessment [314 pages] http://www.efsa.europa.eu/en/consultations/call/130725.htm [accessed 23 August 2013].
- Geens T, Goeyens L, Kannan K, Neels H, Covaci A. 2012. Levels of bisphenol-A in thermal paper receipts from Belgium and estimation of human exposure. Sci Total Environ 435-436:30-33.
- Gillespie B, Chen Q, Le SY, Hong B, Garabrant D, Hedgeman E, Sima C, Lepkowski J, Olson K, Luksemburg W. 2005. A Comparison of Data Analysis Options when Some Values are Below the Limit of Detection (LOD), poster presentation at Dioxin 2006 Conference: August 2006, Oslo, Norway <a href="http://www.sph.umich.edu/dioxin/PDF/OsloPosters/poster\_%20belowLOD.pdf">http://www.sph.umich.edu/dioxin/PDF/OsloPosters/poster\_%20belowLOD.pdf</a> [accessed 13 September, 2014].
- Hormann AM, Vom Saal FS, Nagel SC, Stahlhut RW, Moyer CL, Ellersieck MR, Welshons WV, Toutain PL, Taylor JA. 2014. Holding thermal receipt paper and eating food after

- using hand sanitizer results in high serum bioactive and urine total levels of bisphenol A (BPA). PLoS ONE 9(10):e110509.
- Kaddar N, Harthe C, Dechaud H, Mappus E, Pugeat M. 2008. Cutaneous penetration of bisphenol A in pig skin. J Toxicol Environ Health A 71(8):471-473.
- Lassen C, Mikkelsen SH, Brandt UK. 2011. Migration of bisphenol A from cash register receipts and baby dummies. Survey of Chemical Substances in Consumer Products, No. 110 2011. Danish Ministry of the Environment. <a href="http://www2.mst.dk/udgiv/publications/2011/04/978-87-92708-93-9.pdf">http://www2.mst.dk/udgiv/publications/2011/04/978-87-92708-93-9.pdf</a> (accessed 8 July 2013).
- Liao C, Kannan K. 2013. Concentrations and profiles of bisphenol A and other bisphenol analogues in foodstuffs from the United States and their implications for human exposure. J Agric Food Chem 61(19):4655-4662.
- Liao C, Kannan K. 2014. A survey of alkylphenols, bisphenols, and triclosan in personal care products from China and the United States. Arch Environ Contam Toxicol 67(1):50-59.
- Liao C, Kannan K. 2011. Widespread occurrence of bisphenol A in paper and paper products: implications for human exposure. Environ Sci Technol 45(21):9372-9379.
- Liao C, Liu F, Alomirah H, Loi VD, Mohd MA, Moon HB, Nakata H, Kannan K. 2012a. Bisphenol S in urine from the United States and seven Asian countries: occurrence and human exposures. Environ Sci Technol 46(12):6860-6866.
- Liao C, Liu F, Guo Y, Moon HB, Nakata H, Wu Q, Kannan K. 2012b. Occurrence of eight bisphenol analogues in indoor dust from the United States and several Asian countries: implications for human exposure. Environ Sci Technol 46(16):9138-9145.
- Liao C, Liu F, Kannan K. 2012c. Bisphenol S, a new bisphenol analogue, in paper products and currency bills and its association with bisphenol A residues. Environ Sci Technol 46(12):6515-6522.
- Liao C, Liu F, Moon HB, Yamashita N, Yun S, Kannan K. 2012d. Bisphenol analogues in sediments from industrialized areas in the United States, Japan, and Korea: spatial and temporal distributions. Environ Sci Technol 46(21):11558-11565.
- Longnecker MP, Harbak K, Kissling GE, Hoppin JA, Eggesbo M, Jusko TA, Eide J, Koch HM. 2013. The concentration of bisphenol A in urine is affected by specimen collection, a preservative, and handling. Environ Res 2013 Jul 27:doi 10.
- Lu SY, Chang WJ, Sojinu SO, Ni HG. 2013. Bisphenol A in supermarket receipts and its exposure to human in Shenzhen, China. Chemosphere 92(9):1190-1194.
- Lunder S, Andrews D, Houlihan J. 2010. Synthetic estrogen BPA coats cash register receipts. Environmental Working Group (EWG). Available: <a href="http://www.ewg.org/bpa-in-store-receipts">http://www.ewg.org/bpa-in-store-receipts</a> [accessed 1 November 2012].

- Marquet F, Payan JP, Beydon D, Wathier L, Grandclaude MC, Ferrari E. 2011. In vivo and ex vivo percutaneous absorption of [14C]-bisphenol A in rats: a possible extrapolation to human absorption? Arch Toxicol 85(9):1035-1043.
- Mendum T, Stoler E, VanBenschoten H, Warner JC. 2011. Concentration of bisphenol A in thermal paper. Green Chemistry Letters and Reviews 4(1):81-86.
- Morck TJ, Sorda G, Bechi N, Rasmussen BS, Nielsen JB, Ietta F, Rytting E, Mathiesen L, Paulesu L, Knudsen LE. 2010. Placental transport and in vitro effects of Bisphenol A. Reprod Toxicol 30(1):131-137.
- National Toxicology Program (NTP). 2008. NTP-CERHR Monograph on the Potential Human Reproductive and Developmental effects of Bisphenol A. NTP CERHR Monograph Series(22):v, vii-ix, 1-64 passim <a href="http://ntp.niehs.nih.gov/ntp/ohat/Bisphenol/Bisphenol.pdf">http://ntp.niehs.nih.gov/ntp/ohat/Bisphenol/Bisphenol.pdf</a> [accessed 23 July 2013].
- Östberg T, Noaksson E. 2010. Bisfenol A in Svenska Kvitton. Analysresultat. Institutet för Tillämoa Grön Kemi, Jämtlands läns Landstingb [in Swedish] . <a href="http://www.jegrelius.se/wp-content/uploads/2014/01/Bisfenol-A-i-svenska-kvitton-Jegrelius-101013-pdf-version.pdf">http://www.jegrelius.se/wp-content/uploads/2014/01/Bisfenol-A-i-svenska-kvitton-Jegrelius-101013-pdf-version.pdf</a> (accessed 8 July 2013).
- Porras SP, Heinälä M, Santonen T. 2014. Bisphenol A exposure via thermal paper receipts. Toxicol Lett 230(3):413–420.
- Schreder E. 2010. On the Money: BPA on Dollar Bills and Receipts. Washington Toxics Coalition. Available: <a href="http://watoxics.org/publications/on-the-money">http://watoxics.org/publications/on-the-money</a> [accessed 1 November 2012].
- Teeguarden J, Hanson-Drury S, Fisher JW, Doerge DR. 2013. Are typical human serum BPA concentrations measurable and sufficient to be estrogenic in the general population? Food Chem Toxicol 62:949-963.
- Teeguarden JG, Calafat AM, Ye X, Doerge DR, Churchwell MI, Gunawan R, Graham MK. 2011. Twenty-four hour human urine and serum profiles of bisphenol a during high-dietary exposure. Toxicol Sci 123(1):48-57.
- Twaddle NC, Churchwell MI, Vanlandingham M, Doerge DR. 2010. Quantification of deuterated bisphenol A in serum, tissues, and excreta from adult Sprague-Dawley rats using liquid chromatography with tandem mass spectrometry. Rapid Commun Mass Spectrom 24(20):3011-3020.
- U.S. Environmental Protection Agency (US EPA). 2014. Bisphenol A alternatives in thermal paper (Final Report January 2014). Available: <a href="http://www2.epa.gov/saferchoice/publications-bpa-alternatives-thermal-paper-partnership">http://www2.epa.gov/saferchoice/publications-bpa-alternatives-thermal-paper-partnership</a> [accessed 31 March 2014].
- World Health Organization (WHO). 2011. Toxicological and Health Aspects of Bisphenol A. Report of Joint FAO/WHO Expert Meeting 2–5 November 2010 and Report of Stakeholder Meeting on Bisphenol A 1 November 2010 in Ottawa, Canada

Advance Publication: Not Copyedited

(http://www.who.int/foodsafety/chem/chemicals/bisphenol/en/index1.html accessed 27, November 2012).

Ye X, Zhou X, Hennings R, Kramer J, Calafat AM. 2013. Potential external contamination with bisphenol A and other ubiquitous organic environmental chemicals during biomonitoring analysis: an elusive laboratory challenge. Environ Health Perspect 121(3):283-286.

Environ Health Perspect DOI: 10.1289/ehp.1409427 Advance Publication: Not Copyedited

 Table 1. Receipt characteristics

Receipt category	n	BPA content	BPS content	BPSIP content
		(mg/g paper*)	(mg/g paper*)	(mg/g paper*)
BPA	33	$19.6 \pm 4.7 \text{ (mean } \pm \text{SD)}$	2/34 (6%) > LOD	0/33 (0%) > LOD
		19.3 median	max = 1.09	
		7.0 -36.0 range		
BPS	32	1/32 (3%) > LOD	$15.0 \pm 2.6 \text{ mean} \pm \text{SD}$	0/32 (0%) > LOD
		max = 0.81	14.6 median	
			11.9-26.2 range	
			-	
BPSIP	12	1/12 (8%) > LOD	6/12 (50%) > LOD	$13.5 \pm 0.9 \text{ mean} \pm \text{SD}$
		max = 0.70	max = 0.05	13.9 median
				12.4 -14.8 range
non-cashiers	25	n/a	n/a	n/a

<sup>\*</sup>Divide by 10 to convert mg/g paper to percent of paper weight. LODs were 0.2 mg BPA/g paper, 0.02 mg BPS/g paper, and 0.07 mg BPSIP/g paper.

n/a = not applicable

*Environ Health Perspect* DOI: 10.1289/ehp.1409427 Advance Publication: Not Copyedited

Table 2. Demographic characteristics of study participants

Receipt category	n	sex	age, years	BMI	race
		(% male)	mean ± SD	mean ± SD	
			median (range)	median (range)	
BPA	33	20.6%	$35.0 \pm 12.7$	$29.0 \pm 5.8$	48% black; 39% white; 3% Asian; 9% multiple
			30.1 (19.8-65.0)	27.8 (20.1-43.0)	
BPS	32	41.9%	$35.9 \pm 14.4$	$29.9 \pm 8.0$	38% black; 50% white; 3% Asian; 6% multiple;
			33.2 (19.8-77.5)	27.1 (18.0-46.0)	3% unknown
BPSIP	12	50.0%	$40.4 \pm 13.6$	$26.5 \pm 5.4$	25% black; 58% white; 17% multiple
			40.7 (22.5-60.6)	25.1 (19.0-35.1)	-
non-cashiers	25	60.0%	$44.9 \pm 12.4$	$27.9 \pm 5.0$	24% black; 60% white; 16% unknown
			51.3 (23.1-63.9)	28.0 (19.8-38.6)	

Additional medical history information such as menopausal status, smoking, alcohol use, medications and disease status available in Table S1.

*Environ Health Perspect* DOI: 10.1289/ehp.1409427 Advance Publication: Not Copyedited

**Table 3.** Urine total BPA, BPS, and BPSIP in cashiers and non-cashiers (μg/g creatinine)

	Cashiers, BPA receipts	Cashiers, BPS receipts	Cashiers, BPSIP receipts	Non-cashiers
	n = 33	n = 31	n = 12	n = 21
Compound	geometric mean (SD) [range]	geometric mean (SD) [range]	geometric mean (SD) [range]	geometric mean (SD) [range]
BPA urine				
Cashiers, pre-shift	1.89 (3.63)	1.33 (2.89)	0.71 (2.85)	NA
	[ <lod 57.56]<="" td="" –=""><td>[0.19 - 41.22]</td><td>[&lt; LOD - 2.80)</td><td></td></lod>	[0.19 - 41.22]	[< LOD - 2.80)	
Cashiers, post-shift	2.76 (3.53)**	1.35 (2.34)	1.07 (2.01)	NA
	[0.44 - 187.96]	[0.29 - 20.38]	[0.37 - 4.41]	
Non-cashiers	NA	NA	NA	1.25 (1.79)
				[ <lod 4.19]***<="" td="" –=""></lod>
BPS urine				
Cashiers, pre-shift	0.31 (3.64)	0.23 (3.89)	0.38 (3.75)	NA
•	[ <lod 4.36]<="" td="" –=""><td>[<lod 3.99]<="" td="" –=""><td>[&lt; LOD - 2.16]</td><td></td></lod></td></lod>	[ <lod 3.99]<="" td="" –=""><td>[&lt; LOD - 2.16]</td><td></td></lod>	[< LOD - 2.16]	
Cashiers, post-shift	0.25 (3.16)	0.54 (3.62)*	0.28 (3.06)	NA
	[0.13 - 3.48]	[0.53 - 9.50]	[< LOD - 3.47]	
Non-cashiers	NA	NA	NA	0.41 (5.26)
				[< LOD - 11.04]
BPSIP urine				
Cashiers, pre-shift	4/33 (12.1%)	6/32 (18.8%)	10/12 (83.3%) **	NA
	[all <loq]< td=""><td>[all <loq]< td=""><td>[<lod 0.272]<="" td="" –=""><td></td></lod></td></loq]<></td></loq]<>	[all <loq]< td=""><td>[<lod 0.272]<="" td="" –=""><td></td></lod></td></loq]<>	[ <lod 0.272]<="" td="" –=""><td></td></lod>	
Cashiers, post-shift	6/33 (18.2%)	9/32 (28.1%)	9/12 (75.0%) **	NA
•	[< LOD - 0.035]	[< LOD - 0.762]	[ <lod 1.19]<="" td="" –=""><td></td></lod>	
Non-cashiers	NA	NA	NA	8/25 (32.0%)
				[< LOD - 0.139]

Urine LODs: BPA (0.07 – 0.25 ng/ml), BPS (0.01 – 0.02 ng/ml), BPSIP (0.01-0.02 ng/ml)

<sup>\*</sup> Significant difference between pre vs post (p-value <0.001)

<sup>\*\*</sup> Significant difference compared to non-cashiers (p <0.02)

<sup>\*\*\*</sup>For comparison, the geometric mean level of BPA from NHANES 2011-2012 is 1.72 g/g creatinine (CDC 2015) n/a = not applicable

Environ Health Perspect DOI: 10.1289/ehp.1409427 Advance Publication: Not Copyedited

Table 4. Serum total BPA, BPS, and BPSIP in cashiers

Compound	cashiers, BPA receipts (number >LOD)	cashiers, BPS receipts (number >LOD)	cashiers, BPSIP receipts (number >LOD)
BPA serum			
pre-shift	7/33(21.2%)	4/32 (12.5%)	4/12 (33.3%)
post-shift	7/33 (21.2%)	4/32 (12.5%)	0/12 (0%)
BPS serum			
pre-shift	9/33 (27.3%)	5/32 (15.6%)	2/12 (16.7%)
post-shift	5/33 (15.2%)	13/32 (40.6%)*	1/12 (8.3%)
BPSIP serum			
pre-shift	9/21 (42.9)	5/15 (33.3%)	7/12 (58.3%)
post-shift	6/17 (35.3)	7/16 (43.8%)	6/12 (50.0%)

Serum LODs: BPA (0.045–0.35 ng/ml), BPS (0.002 – 0.01 ng/ml), BPSIP (0.005 - 0.008 ng/ml)

<sup>\*</sup> Significant difference between pre vs post (p-value = 0.02)

Advance Publication: Not Copyedited

# Figure legends

**Figure 1.** Chemical structures, common names, systematic names, molecular formulas and CAS numbers of BPA, BPS, and BPSIP

**Figure 2.** Pre- and post-shift urinary levels of BPA and BPS: Individual patterns and group median (25%-75%). Error bars for the group medians indicate the 25–75% range. \*Significant difference between pre vs post (p-value <0.001)

# Figure 1

BPSIP (D-8) 4-Hydroxyphenyl 4-isoprooxyphenylsulfone  $\rm C_{15}H_{16}O_4S$  CASRN: 95235-30-6

Figure 2

